(2) A sample of no less than 40 milliliters of the final product distributed in approximately equal amounts into four final containers.

(3) The product shall not be issued by the manufacturer until written notification of official release of the lot is received from the Director, Center for Biologics Evaluation and Research.

[38 FR 32064, Nov. 20, 1973, as amended at 42 FR 27582, May 31, 1977; 48 FR 13025, Mar. 29, 1983; 49 FR 23834, June 8, 1984; 51 FR 15610, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

## Subpart D—Cholera Vaccine

### § 620.30 Cholera Vaccine.

The proper name of this product shall be Cholera Vaccine, which shall consist of an aqueous preparation of equal parts of Ogawa and Inaba serotypes of killed *Vibrio cholerae* bacteria.

[41 FR 18295, May 3, 1976]

### § 620.31 Production.

(a) *Strains of bacteria.* (1) A strain of Ogawa and a strain of Inaba serotypes of *V. cholerae* shall be used in the manufacture of the vaccine. Each serotype strain shall have been shown in controlled field studies to yield a vaccine no less potent than vaccines prepared from Ogawa strain 41 and Inaba strain 35A3 obtained from the Center for Biologics Evaluation and Research.

(2) Antigenic integrity of the strains shall be verified by (i) the agglutination of living bacteria of each serotype by cholera O Group I antiserum; (ii) the agglutination of the Ogawa strain in monospecific Ogawa antiserum and of the Inaba strain in monospecific Inaba antiserum; and (iii) the absence of spontaneous agglutination of living bacteria of either strain in 0.85 percent sodium chloride solution during incubation for at least 5 hours at 37° C.

(b) *Propagation of bacteria.* The culture medium for the propagation strains shall not contain ingredients known to be capable of producing allergenic effects in human subjects. The harvested bacteria shall be free of extraneous bacteria, fungi, and yeasts as demonstrated by microscopic examination and cultural methods. Bacteria of the two serotypes shall be grown separately.

(c) *Bacterial content.* (1) The number of bacteria in each separate bacterial harvest shall be determined by use of the U.S. Opacity Standard not later than 2 hours after harvest and before treatment with a preservative or other agent capable of altering opacity of the bacterial suspension.

(2) The vaccine shall contain equal numbers of bacteria of the Ogawa and Inaba serotypes, and the total number shall not exceed $8 \times 10^9$ bacteria per milliliter.

(d) *Nitrogen content.* The total nitrogen content of the vaccine shall not exceed 0.3 milligram per milliliter for bacteria grown on solid medium or 1.0 milligram per milliliter if grown in liquid medium. In no instance shall the vaccine contain more than 0.07 milligram per milliliter of nitrogen precipitable by the addition of an equal volume of 10 percent trichloracetic acid.

(e) *Preservative.* The vaccine shall contain a preservative.

[41 FR 18295, May 3, 1976, as amended at 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

### § 620.32 U.S. Standard preparations.

The following U.S. Standard preparations shall be obtained from the Center for Biologics Evaluation and Research, Food and Drug Administration, for use as prescribed in this subpart:

(a) *Vaccine standard.* The U.S. Standard Cholera Vaccine, Ogawa serotype, and U.S. Standard Cholera Vaccine, Inaba serotype, shall be reconstituted as directed for determining the potency of Cholera Vaccine.

(b) *Opacity standard.* The U.S. Opacity Standard for use in estimating the bacterial content of the vaccine and of the challenge culture.

(c) *Seed culture.* Seed cultures of *V. cholerae,* Inaba serotype, strain 35A3 and Ogawa serotype, strain 41, for preparation of vaccine challenge cultures for use in the vaccine potency test.

[41 FR 18295, May 3, 1976, as amended at 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

### § 620.33 Potency tests.

Each lot of vaccine shall be subjected to two potency tests. One test shall determine the potency of the vaccine in comparison with the U.S. Standard